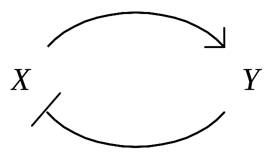
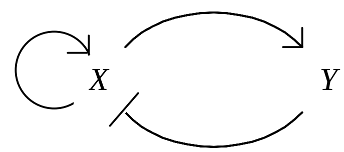
Problem Set 3 **Assigned: 10.19.04**
Due in class **Due: 11.02.04**

Several protein expression levels in plant and animal cells go through a daily cycle, driven by exposure to sunlight during the day and darkness at night. However, even in complete darkness, these expression levels oscillate with an intrinsic period of about 24 hours. The systems which drive these oscillations are known as *circadian clocks*. At the heart of most of these systems is a pair of transcriptionally regulated proteins: an activator (*X*) and an inhibitor (*Y*). In this problem set, we will see how such a simple system can be made to generate oscillations. We consider two possible system architectures (arrows represent activation, blunt ends represent inhibition):

(A)



(B)



The corresponding dynamical equations are (using $x = [X]$ and $y = [Y]$):

(A)
$$\frac{dx}{dt} = v_x + k_x \frac{A_1}{A_1 + y} - \gamma_x x$$
$$\frac{dy}{dt} = v_y + k_y \frac{x}{A_2 + x} - \gamma_y y$$

(B)
$$\frac{dx}{dt} = v_x + k_x \frac{x^2}{A_3^2 + x^2}\frac{A_1}{A_1 + y} - \gamma_x x$$
$$\frac{dy}{dt} = v_y + k_y \frac{x}{A_2 + x} - \gamma_y y \; .$$

1. Biochemical interpretation of dynamical equations

(10) *a.* Identify the parameters corresponding to basal transcription rate, maximal transcription rate, and degradation rate.

(10) *b.* We have assumed the following: for both (A) and (B), the *X* promoter is inactivated by the binding of a single molecule of *Y*, and the *Y* promoter is activated by the binding of a single molecule of *X*. In addition, for (B), the *X* promoter is activated by the cooperative binding of two molecules of *X*. The various fractions that appear in the dynamical equations represent the fractions of promoters that are active under these conditions. Give a biochemical interpretation of each of these fractions.

2. Normalization of units. Assume that $A_2 >> x$. Define new time and concentration units so that $\bar{t} = \gamma_y t$, $\bar{x} = x / A_3$, and $\bar{y} = y / A_1$. The dynamical equations can then be written in the following form:

(A)
$$\frac{d\bar{x}}{d\bar{t}} = \bar{\gamma}_x (\bar{v}_x + \bar{k}_x \frac{1}{1+\bar{y}} - \bar{x})$$
$$\frac{d\bar{y}}{d\bar{t}} = \bar{v}_y + \bar{k}_y \bar{x} - \bar{y}$$

(B)
$$\frac{d\bar{x}}{d\bar{t}} = \bar{\gamma}_x (\bar{v}_x + \bar{k}_x \frac{\bar{x}^2}{1+\bar{x}^2}\frac{1}{1+\bar{y}} - \bar{x})$$
$$\frac{d\bar{y}}{d\bar{t}} = \bar{v}_y + \bar{k}_y \bar{x} - \bar{y} \; .$$

(10) *a.* Calculate the values of the new (barred) parameters in terms of the old parameters.

(10) *b.* Discuss whether these equations can be further simplifed by normalization of units.

From now on we will work with the simplified equations, dropping the bars on our variable and parameter symbols. These equations are of the general form

$$dx/dt = f(x,y)$$
$$dy/dt = g(x,y).$$

Choose one of the following two problems.

3. Global dynamics. Assume the following parameter values: $v_x = 0.1$; $v_y = 0.0$; $k_x = 4.0$; $k_y = 2.0$; $\gamma_x = 10.0$. Do this problem for systems (A) and (B) separately.

(30) *a.* On a graph of *y* vs. *x*, plot the nullclines $f(x,y) = 0$ and $g(x,y) = 0$. You should find that the nullclines intersect only once, dividing the graph into four regions. For each of these regions, draw a few arrows indicating the direction of motion (e.g. if $dx/dt < 0$ and $dy/dt > 0$, the arrows point NW). Comment on the stability of the fixed point.

(30) *b.* Write a MATLAB program to solve the dynamical equations upto $t = 20.0$. Plot the output on a graph along with the nullclines. You should find that system (A) does not oscillate, while system (B) does. It turns out that autoactivation by *X* is crucial to the generation of oscillations. Problem 4 examines the reasons why this is so.

4. Stability analysis. Let $\{x_0, y_0\}$ be a fixed point of the system (so that $f(x_0,y_0) = 0$, $g(x_0,y_0) = 0$). Define the matrix

$$A = \left.\begin{bmatrix} \delta f/\delta x & \delta f/\delta y \\ \delta g/\delta x & \delta g/\delta y \end{bmatrix}\right|_{x_0,y_0}$$

Recall that the fixed point is stable if and only if Tr $A < 0$ and Det $A > 0$.

(20) *a.* For system (A), prove that the system will always converge to a stable fixed point.

(20) *b.* For system (B), assume the following parameter values: $v_x = 0.1$; $v_y = 0.0$; $k_x = 4.0$; $k_y = 2.0$. Let γ_x be a free parameter.

Find the fixed point of the system numerically. The matrix *A* evaluated at the fixed point should be a function of γ_x alone. Oscillations will arise whenever this fixed point becomes unstable. Write down the conditions on γ_x under which the system is oscillatory. (This transition from a stable to an oscillatory system is known as a Hopf bifurcation.)

(20) *c.* For system (B), write a MATLAB program to solve the dynamical equations using the parameters given in part *b*. Do this for two values of γ_x, one of which gives an oscillatory system, and the other a stable one. On the same graphs plot out the nullclines $f(x,y) = 0$ and $g(x,y) = 0$.

(0) *d.* CHALLENGE. In the limit $\gamma_x >> 1$, estimate the period of system oscillations.